

## Effect of Different Age of Pepper Seeds on the Vegetative Behaviors and Physiological Status of Seedlings

Nikolay Panayotov<sup>1</sup>, Nevena Stoeva<sup>1</sup>

<sup>1</sup>*Agricultural University-Plovdiv, Plovdiv, Bulgaria*

### Abstract

The aim of the present study was to evaluate the influence of the different age of pepper seeds on the changes of vegetative development and physiological status of the grown seedlings. Experiments were carried out with two pepper cultivars. The seeds were stored for 120 months in ambient conditions. Through 12 months the sowing quality was established. The vegetative experiments were conducted. Morphological behaviors, an intensity of respiration and guaiacol peroxidase enzyme activity in seeds and the leaf gas-exchange were measured. Pepper seeds preserve normal sowing quality for up to 4 years. The length of root and stem decrease during storage. The leaf-gaze exchange was inhibited for seedlings of aged seeds.

*Key words:* sowing quality, germination, leaf gas-exchange, photosynthesis, transpiration

### Introduction

The quality and the changes occurring during storage of vegetable seeds are extremely important for agricultural science and practice (Black et al, 2008). Penalozza et al. (2005) believe that the high quality of seeds is the basis for the development of normal plants and emphasize that for objective assessment, it is necessary to trace these indicators. Many authors reported that during long-term storage, the seeds lost their viability and therefore the evaluation of seed deterioration over time is required (Hay and Probert, 2013; Treuren et al., 2013).

Pepper is characterized by a relatively short period of maintaining a good sowing quality (Panayotov 2010). According to Ozoban and Demir (2002), the loss of vitality is increased when the pepper seeds' humidity is over 6%. In experiments with pepper seeds, Kaewnaree et al. (2011) found out a reduction of germination during storage, accompanied by a number of changes in physiological indicators, mostly related to peroxidase and antioxidant activity.

The main aim of the present study was to monitor the influence of long-term storage of pepper seeds on the changes in vegetative development and on the physiological condition of the seedlings obtained from these seeds.

## Material and Methods

The experiments were conducted at the Department of Horticulture and the Department of Physiology and Biochemistry of Plants at the Agricultural University of Plovdiv, Bulgaria with seeds of pepper varieties Kurtovska kapia 1619 and Bulgarski rotund, stored since 2004 in paper bags for a period of 120 months under ambient conditions with initial moisture 6.0-6.9%. Every 12 months, analysis of the vitality status of seeds and plant development was performed. The germinating energy (G.E.) and germination (G.) of seeds were analyzed (ISTA, 2003). Respiratory rate activity in  $\text{mg CO}_2 \text{ g}^{-1} \text{ h}^{-1}$  (by infrared gas analyzer LCA-4) and activity of the enzyme guaiacol peroxidase in  $\text{U g}^{-1} \text{ FM}$  (Bergmeyer et al, 1974) of imbibed seeds was determined in three replicates. The vegetation experiments were carried out in growth-chamber under controlled conditions. 100 seeds were sown in pods № 10 in the peat-pearlite mixture in ratio 3:1 in four replicates. With regards to the percentage of plants developed, at least two true leaves were determined. The vegetative behaviors - length and weight of roots, height and weight of stems, number, weight and size of leaves (determined by an electronic digital area meter NEO-2 (Kerin et al., (1997) were established on 15 plants, 50 days after germination. The parameters of leaf gas exchange: stationary photosynthesis ( $\mu\text{mol/m}^2\text{s}^{-1}$ ), intensity of transpiration ( $\text{mmol m}^{-2}\text{s}^{-1}$ ) and stomata conductivity ( $\text{mol m}^{-2} \text{ s}^{-1}$ ) on first fully developed leaves of the plants top, in five-time replicability, through photosynthetic system LCA-4 were determined. The statistical analysis ANOVA was performed.

## Results and Discussion

Aging of pepper seeds caused significant changes in their vital status. A sharp reduction in germination energy (Table 1) was observed for variety Kurtovska kapia 1619 in the fourth year, and for Bulgariski rotund in the sixth one, when germinated seeds were below 30%.

Insignificant values for this indicator were reported after the seventh year of storage. The seeds produced normal germination (around 70%) in 4-5 year. Comparatively, the high percentage was observed in seeds from the first, second and third year. The sharpest decrease of germination began in the seventh year, with 37.65% for the first cultivar and 43.63% for the other one, towards the initial values (assumed to be 100%). Most of the differences between the cultivars are with statistical significance.

According to Copeland and Mc Donald (2001), the standard test for germination does not give a complete picture of the behaviors of the seed lot under field conditions. The percentage of developed plants gradually decreased, such as up to four years it is in the range from 56.6% to 71.2%. Only between 6.8% (Kurtovska kapia 1619) to 14.4% (Bulgarski rotund) of decade-stored seeds have developed normal plants. The hypothetical model of Delouche and Baskin (1973) points out that one of the primary changes in degradation is a disturbance in the intensity of their respiration and biosynthesis. Yong-Bi et al. (2015) also found that seed ageing is associated with the many physiological changes. The stored pepper seeds exhibit a different intensity of respiration. The dynamics of change is similar for both varieties. It is noted that, up to the fourth year the respiration does not change significantly, but then it gradually weakness.

Tab. 1. Viability of pepper seeds depends on age and percentage of developed plants. Intensity of respiration (I. R.) and activity of enzyme guaiacol peroxidase (E. A.) in seeds

*Виталност сјемена паприке у зависности од старости и процента развијених биљака. Интензитет дисања и активности ензима гвајакол пероксидазе у сјеменима*

Years / године	Kurtovska kapia 1619					Bulgarski rotund				
	G. E. (%)	G. (%)	D. P. (%)	I.R.	E.A.	G. E. (%)	G. (%)	D. P. (%)	I.R.	E.A.
1	66.67	83.33	68.7	6.15	0.62	70.0	86.33	71.2	6.00	0.66
2	46.67	82.0	66.6	6.36	0.63	58.67	82.33	68.4	5.88	0.68
3	32.0	80.0	64.3	6.43	0.70	50.67	80.34	63.7	5.66	0.59
4	28.67	79.33	60.8	6.20	0.70	40.67	70.0	56.6	5.80	0.53
5	26.00	76.67	57.8	5.43	0.58	36.67	64.0	40.4	5.22	0.50
6	24.67	66.67	45.9	4.96	0.42	22.0	62.0	36.3	4.80	0.44
7	10.67	52.0	40.0	4.31	0.28	7.33	48.67	30.4	4.22	0.32
8	1.33	45.33	32.3	4.22	0.22	2.0	42.67	25.8	3.22	0.23
9	2.00	37.33	26.8	3.20	0.18	0.67	41.33	18.6	2.90	0.18
10	0.0	10.67	6.8	3.30	0.11	0.0	24.0	14.4	2.65	0.13
p=0.05	17.23	15.73	18.1	1.01	0.24	15.83	11.15	21.3	1.45	0.31

At the end of the storage period, the respiration on the seeds of Kurtovska kapia 1619 was 51.6% for the first year seeds, while for Bulgarski rotund it was - 44.0%. According to Li et al. (2005), the main mechanism for the aging of pepper seeds is associated with increased peroxidation.

Panayotov (2016) emphasized that in the imbibed seed the enzyme activity increases. These features were observed only in the early periods of storage mostly for the seeds of Kurtovska kapia 1619. In this cultivar, the change in activity of the enzyme guaiacol peroxidase marked a slight activation in 4 years of storage. Sharp decrease established in the 7<sup>th</sup> year and to the end of the storage it was 17.4% in comparison to one-year seeds. For the other cultivar, enzyme activity has a significant decrease in the 7<sup>th</sup> and 8<sup>th</sup> year. At the end of storage, it was 19.66%. Copeland and Mc Donald (2001) reported that maybe this activity affects most the degradation process. The data are with statistical significance. Some authors (Schmidt, 2000; Panayotov, 2010) point out that besides vitality, extremely important sign is the morphological development of seedlings, indicating that the seeds do not only germinate, but can also form normal plants.

The length of the root (Fig. 1) decreased regularly through all the years of storage. In the first cultivar from 6.5 cm plants of one-year stored seeds, in the tenth year they reached up to 2.1 cm. A similar trend is observed in the other cultivar. In both cultivars comparatively the weight evenly reduces and, at the end of the storage, it was only 20.8% and 19.6% compared to the initial measurements, for Kurtovska kapia 1619 and Bulgarski rotund respectively.

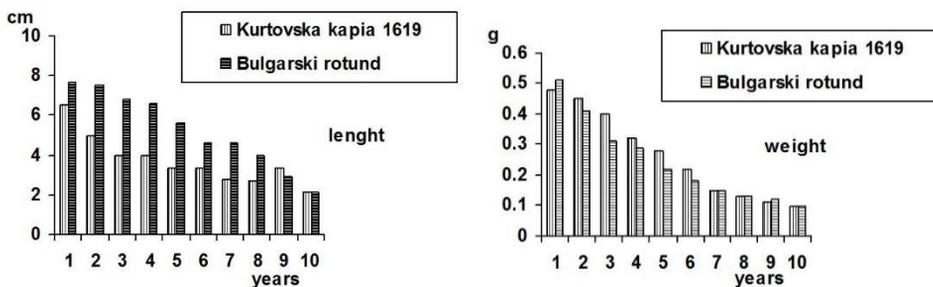


Fig.1. Морфолошке манифестације корена  
*Морфолошке манифестације корена*

More significant are the changes in the height of the stem (Fig. 2), as the strongest decrease was observed in plants grown from 4-year-old seeds of the cultivar of kapia type (43% from the first year) and in plants from seeds of 6 years for the other cultivar (54% from the initial values).

The decrease between the initial value and that of the last year of storage was 3.75 times for Kurtovska peppers 1619 and 2.65 times for the other cultivar.

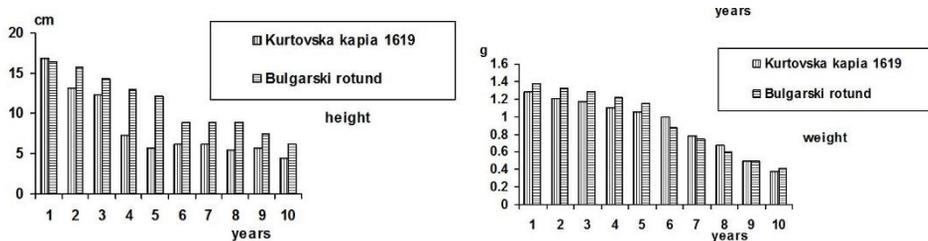


Fig. 2. Vegetative characteristics of stem  
*Вегетативне карактеристике стабла*

A similar development was observed in stem weight. More dramatical was the decrease in the plants of 6 and 7-year-old seeds. The difference between the initial and final measurements in both cultivars was 3.36 times. Data on the number of leaves and photosynthetic leaf area are shown in Figure 3 and Figure 4. In both cultivars, the dynamics in these characteristics is different. In the first cultivar, the changes are gradual in direction of inhibition in the number and area of leaves, and after 10 years of storage, these parameters are approximately 20% and 10% of those of annual seeds. In Bulgarski rotund, only after ten-year seeds, a significant decrease in these indicators was observed, which was 22% and 21.7% compared to the annual seeds. On one hand this demonstrates a much better storability and high vigour in Bulgarski rotund and on the other hand it is an evidence of strong genotypic response to these indicators. The weight of leaves was also with a clear trend of inhibition, but without a very steady rate. The decrease was particularly strongly manifested after 6 and 7 years of storage of seeds. In plants formed by a ten-year seed, it was 6.1 and 9.8 times less than those from seeds from the first year, for Kurtovska kapia 1619 and Bulgarski round, respectively.

The total vegetative weight of the whole plant gives much clearer idea for overall development (Fig. 5). In both cultivars, the increasing of the age of seeds causes decreases evenly in the fifth year. The more significant decrease was observed in plants developed by the 6-year seeds with 0.38 g and 0.49 g for Kurtovska kapia 1619 and Bulgarski rotund, respectively. The decrease between initial measurements and those at the end of the experiment is approximately 4 times, as with the ten years, the weight of plants for Kurtovska kapia 1619 was only 12.75% compared to those of annual seeds, and for Bulgarski rotund it was 20.87%.

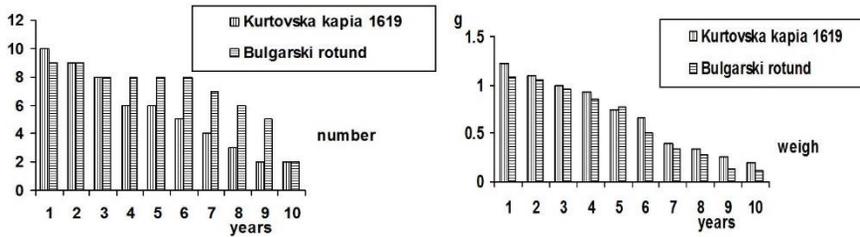


Fig. 3. Morphological features of leaves  
*Морфолошке карактеристике листова*



Fig. 4. Leaf area  
*Лисна површина*



Fig. 5. Total vegetative weight  
*Укупна вегетативна маса*

The existence of such inhibition in total vegetative weight was influenced most by the weight of leaves, where the decrease is approximately 6 times in Kurtovska kapia 1619 and 10 times for Bulgarski rotund, while in the root system and the stem it is 5 and 3 times, respectively. This indicates that leaves are influenced more strongly by the age of the seed from which the plants have formed. After certain ages of storage of pepper seeds, Oladiran and Agunbiade (2000) established that the seedling growth and development decline. They concluded that the seedling development from aged seeds can be taken as an indication for evaluation of pepper seed status. Yan-Ling and Huan-Cheng (2014) also established that the seed aging resulted in reduced seedling growth and vegetative development, which was a consequence of decline in seed reserve depletion percentage.

The parameters of leaf gas exchange (Figures 6,7) can assess in greater fullness the physiological status of the plants and through them could get an idea for their overall development (Berova et al, 2008). Long-term storage period significantly inhibited the leaf gas exchange in plants. Despite the similarity in the dynamics of the process, at the end of the study period, the two cultivars are characterized by differences in terms of their inhibition.

In Kurtovska kapia 1619 the rate of photosynthesis was suppressed by approximately 33% and those of transpiration with 38%. In the second cultivar, the rate of photosynthesis was inhibited more significant and it was only 8% of this of plant formed from annual seeds, while the rate of transpiration was 39%. The state of stomata conductivity in both cultivars follows the similar trend.

According to Matilla et al. (2005), the reason for reducing the intensity of physiological processes most likely is due to the different content of ethylene and abscisic acid that increases their synthesis during aging. The period of growth of these plants is generally quite long, which causes the increase in the amount of these substances.

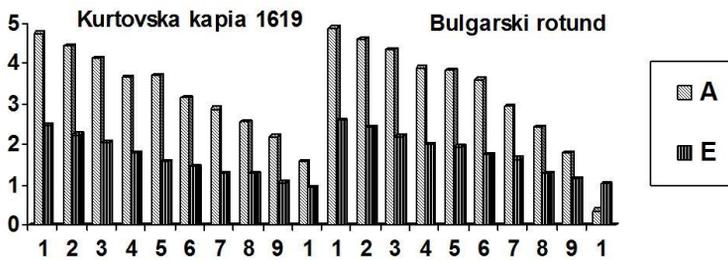


Fig. 6. Intensity of photosynthesis (A) and transpiration (E)  
*Интензитет фотосинтезе и транспирације*

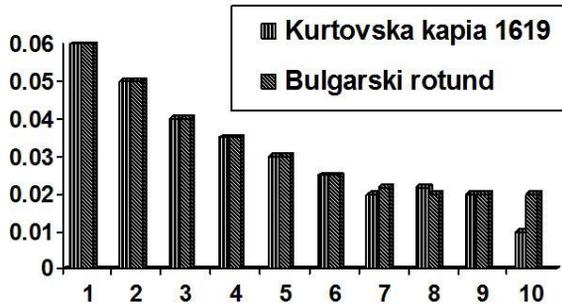


Fig. 7. Stomata conductivity  
*Кондуктивитет стома*

## Conclusion

The seeds of pepper in long-term storage preserve normal germination up to 4-5 years, it decreases sharply after 7 years and the proportion of normal developed plants declines.

The respiration and activity of the enzyme guaiacol peroxidase in the seeds gradually weakens after the fourth year of storage. The weight and length of root system and the height of the stem reduces gradually after 4-6 years of storage. A clearly pronounced genotype response was observed in the number of leaves and leaf area. The weight of leaves is influenced more strongly. Long-term period of storage significantly inhibits the leaf gas exchange in plants. All these parameters can be used for better assessment of the seed deterioration.

## References

- Bergmeyer, H., Gawehn, K. & Grassl, M. (1974). Enzymes as biochemical reagents. In: Bergmeier, H.U. (ed), *Methods in Enzymatic Analysis* (pp. 165-245). New York: Academic Press.
- Berova, M., Stoeva, N., Zlatev, Z. & Ganeva, D. (2008). Physiological response of some tomato genotypes to high temperature stress. *J. of Central European Agriculture*, 9 (4), 723-732.
- Black, M., Bewely, J.D. & Halmer, P. (2008). Seed deterioration. In: Black, M. (ed.), *The Encyclopedia of Seeds Science* (p.156). Oxfordshire (UK): CAB International.
- Copeland, L. & McDonald, M. (2001). Seed storage. In Copeland, L. (ed.), *Principles of Seed Science and Technology* (pp. 220-289). New York: Chapman & Hall.
- Delouche, J.C. & Baskin, C.C. (1973). Accelerate aging techniques for predicting the relative storability of seed lots. *Seed Science and Technology*, (1), 427-452.
- Hay, F.R. & Probert, J. (2013). Advances in seed conservation of wild plant species: a review of recent research. *Conservation Physiology*, 1(1), 42-52.
- ISTA, 2013. *International Rules for Seed Testing*. Zurich (Switzerland): ISTA.
- Kaewnaree, P., Vichitphan, S., Klanrit, P., Siri, B. & Vichitphan, K. (2011). Effect of Accelerated Aging Process on Seed Quality and Biochemical Changes in Sweet Pepper (*Capsicum annum* L.) Seeds. *Biotechnology*, 10 (2), 175-182.
- Kerin, V., Berova, M., Vasilev, A., Stoeva, N. & Zlatev, Zl. (1997). *Plant physiology*. (p. 246). Publishing house of Agricultural University-Plovdiv.
- Li, X., Zou, X. & Liu, Z. (2005). On physiological and biochemical changes of artificially aged pepper seeds. *J. of Hunan Agricultural University*, 31(3), 265-268.

- Matilla, A., Gallardo, M. & Puga-Hermida, M.I. (2005). Structural, physiological and molecular aspects of heterogeneity in seeds. *Seed Science Research*, 15 (2), 63-76.
- Ozoban, M. & Demir, I. (2002). Longevity of pepper seeds in relation to seed moisture and storage temperature. *Indian Journal of Agricultural Sciences*, 72 (10), 589-593.
- Oladiran, J. and Agunbiade, S. (2000). Germination and seedling development from pepper seeds storage in different packaging materials. *Seed science and technology*, 28 (2), 413-419.
- Panayotov, N. (2010). Viability and vigour of pepper seeds during their ageing. *Scientific works, Agricultural University-Plovdiv, LV* (1), 347-352.
- Panayotov, N. (2016). Vigour, vigour tests and sowing potential of vegetable seeds. *Agricultural science, VIII* (20), 7-20.
- Penaloza, P., Ramires-Rosales, G., McDonalds, M. & Bennett, M. (2005). Lettuce (*Lactuca sativa* L.) seed quality evaluation using seed physical attributes, saturated salt accelerated aging and the seed vigour imaging system. *Electronic Journal of Biotechnology*, 8 (3), 297-307.
- Schmidt, L. (2000). *Guide to Handling of Tropical and Subtropical Forest Seed* (p.178). Danida Forest Seed Centre, Denmark.
- Treuren, R., de Groot, E. & Hintum, J. (2013). Preservation of seed viability during 25 years storage under standard genebank conditions. *Genet. Resources Crop Evol.*, 60 (2), 407-421.
- Yan-Ling, Z. & Huan-Cheng, M. (2014). Effects of seed aging on seed germination and seed reserve utilization in mumian. *HortTechnology*, 24 (4), 471-474.
- Yong-Bi, F., Ahmed, Z. & Diederichsen, A. (2015). Towards a better monitoring of seed ageing under *ex situ* seed conservation. *Conservation Physiology*, 3(1), 26-34.

# Утицај различите старости сјемена паприке на вегетативне фазе и физиолошки статус садница

Николај Панајотов, Невена Стојева

*<sup>1</sup>Пољопривредни Универзитет у Пловдиву, Пловдив, Бугарска*

## Сажетак

Примарни циљ овог истраживања био је да се процјени утицај различите старости сјемена паприке на вегетативни раст и физиолошко стање садница. Експерименти су обављени на двије врсте сјемена паприке. Сјемена су била остављена 120 месеци под нормалним условима. Током 12 месеци је контролисан и регистрован квалитет и могућност за њихово сијање. Обављени су вегетативни експерименти. Мјерене су морфолошке појаве, интензитет дисања и размјена гасова у листовима. Сјемена паприке су задржала стандардни квалитет за сјетву у периоду до четири године. Дужина корјена и стабла током складиштења је смањена. Размјена гасова у листовима је смањена код садница добијених из сјемена веће старости.

*Кључне ријечи:* квалитет сјемена, клијање, размјена гасова у листовима, фотосинтеза, испаравање

Nikolay Panayotov  
E-mail address: [nikpan@au-plovdiv.bg](mailto:nikpan@au-plovdiv.bg)

Received: February 24, 2017  
Accepted: July 12, 2017